Biofilm system 4

Monitoring gene expression in microbial biofilms.

Introduction.

The hydrodynamic flow chamber biofilm system is an excellent research tool for monitoring differential gene expression in heterogeneous populations where spatiotemporal information is important.

P. aeruginosa and other Gram-negative bacteria such as *Salmonella typhimurium* and *Escherichia coli* are capable of altering the composition of the outer membrane in response to environmental conditions by adding positively charged N_4-aminoarabinose (4-amino-4-deoxy-L-arabinose) to the 1' and/or 4' phosphate groups of lipid A of the outer membrane LPS molecules. The addition of N_4-aminoarabinose to lipid A makes the net charge of the outer membrane less negative which reduces the affinity of cationic antimicrobial peptides (CAMPs) for the membrane rendering the bacteria less susceptible to cationic antimicrobial peptides such as colistin. In *P. aeruginosa* the genes involved in the incorporation of N_4-aminoarabinose to lipid A are located in a single operon, *arnBCADTEF* (*arnB-operon*). The operon is regulated by two two-component systems, *phoPQ* and *pmrAB* that respond to differences in Mg^{2+} concentration. Interestingly, the *arnB*-operon is induced by the presence of antimicrobial peptides, such as colistin, in the growth environment independently of these systems. The molecular details of this recognition of CAMPs are not completely elucidated and the level of induction varies with the molecular structure of the peptides.

In this exercise we will examine gene expression of the *P.aeruginosa arnBCADTEF* operon and compare that to the expression of the two component regulator operon *pmrAB* in biofilms challenged with the antimicrobial peptide colistin at different concentrations (MIC (= 4 mg/L), 2xMIC (=8 mg/L), 4xMIC (= 16 mg/L) and 25 mg/L). We will use a reporter gene fusion to the *arnB* operon or *pmrAB* promoter that has been inserted in the att Tn7 site on the chromosome of *P.aeruginosa* enabeling monitoring the response of promoter activity within the biofilm to colistin challenge. The cells in the biofilm will also be stained with propidium iodide to indentify dead cells. Moreover, we will use a mutant with constitutive *pmrAB* expression to investigate how that affect colistin resistant sub population formation. It is essential take a look at the paper *Haagensen et.al. J. Bact* (2007) (included in the course folder) for a more extensive background for the experiments.

Aim.

The aim of this exercise is to demonstrate how the hydrodynamic flow chamber biofilm system can be used to monitor gene expression in heterogeneous populations.

There are a few question we would like you to pay extra attention to during the exercise:

Where and how is *arnB* expressed?

Where and how is *pmrAB* expressed?

Is there a correlation in *arnB* and *pmrAB* expression?

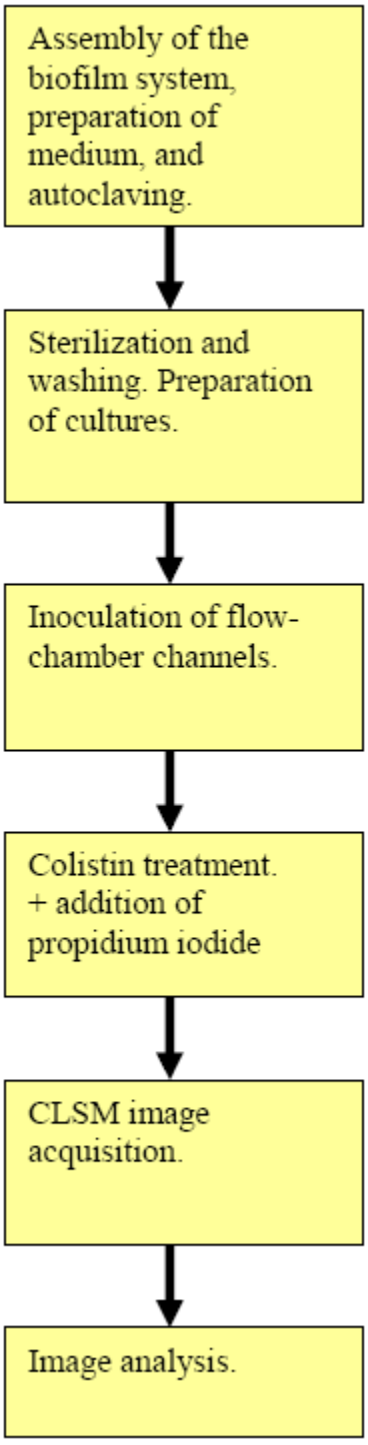
How does colistin concentration affect the *arnB* and *pmrAB* expression pattern in the biofilms?

What factors are affecting the expression?

What are the strengths and weaknesses of monitoring gene expression flow chamber biofilms?

What are the implications of these results on the study of biofilms and biofilm formation in general?

Experimental procedures


Assembly of the biofilm system, preparation of medium, and autoclaving.
Sterilization and washing. Preparation of cultures.
Inoculation of flow-chamber channels.
Colistin treatment. + addition of propidium iodide
CLSM image acquisition.
Image analysis.

Day 1. Assembly of the biofilm system, preparation of medium, and autoclaving.

See the protocol for biofilm work.

Day 2. Sterilization and washing of the biofilm system and saturation of tubings with media over night.

See the protocol for biofilm work.

. Preparation of cultures.

- The strains should be inoculated in 10mL LB and incubated O.N. at 30°C. These cultures will be used to inoculate biofilms on day 4.
- Inoculate the following strains.
    - ***P. aeruginosa*, PA01 *arnB::gfp***
    - ***P. aeruginosa*, PA01, *pmrAB::gfp***
    - ***constitutive mutant X, pmrAB::gfp***

Day 3. Inoculation of flow-chamber channels.

See the protocol for biofilm work.

The O.N. cultures should be diluted 1000 times in 0.9% NaCL before inoculation.

- **channel 1 PA01 *arnB::gfp* (untreated control),**
- **channel 2 PA01, *pmrAB::gfp* (untreated control),**
- **channel 3 *constitutive mutant X* (untreated control),,**
- **channel 4 PA01 *arnB::gfp* (4mg/L colistin),**
- **channel 5 PA01, *pmrAB::gfp* (4mg/L colistin),**
- **channel 6 *constitutive mutant X* (4mg/L colistin)**
- **channel 7 PA01 *arnB::gfp* (8mg/L colistin)**
- **channel 8 PA01, *pmrAB::gfp* (8mg/L colistin)**
- **channel 9 *constitutive mutant X* (8mg/L colistin)**
- **channel 10 PA01 *arnB::gfp* (16mg/L colistin)**

- **channel 11 PA01, *pmrAB::gfp* (16mg/L colistin)**
- **channel 12 *constitutive mutant X* (16mg/L colistin)**
- **channel 13 PA01 *arnB::gfp* (25mg/L colistin)**
- **channel 14 PA01, *pmrAB::gfp* (25mg/L colistin)**
- **channel 15 *constitutive mutant X* (25mg/L colistin)**

Day 6. Colistin treatment.

The 4 day-old PAO1 biofilm tagged with GFP should be treated with the antibiotic colistin for 20 h. This is done by changing the medium irrigating the flow channels of interest to medium containing colistin in a final concentration as indicated above. Addition of 10 µl Propidium iodide to 1 L media (PI, 20mM/ml in DMSO) as Live/Dead indicator. Exchange also the medium in the bubble traps. (This is done by removing the orange caps from the bubble trap and turning the pump speed to max (90 rpm) for 1.5-2min. Remember to turn the speed to 1.75 rpm before recapping the bubble traps.)

Note that PI is light sensitive (-> Cover the whole biofilm system with silver foil. The colistin treatment use about 350 ml of medium per chamber (= 3 channels) per 24 hours).

Day 7, morning. CLSM image acquisition.

All microscopic observations are performed by the use of a Zeiss LSM510 Confocal Laser Scanning Microscope (CLSM) equipped with lasers, filter sets, and detectors for simultaneous monitoring of Gfp (excitation 488nm, emission 517nm) and red-fluorescence emitted from the PI (excitation 543nm, emission 565nm); The images are acquired with a 40x/1.3 Plan-Neofluar oil immersion objective. The untreated chamber will be stained with living cell indicator dye Syto9 which is a green fluorescent stain.

Image analysis.

Prepare images of the different biofilms using Imaris software.